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The effects of 17-α-ethyl-19-nortestosterone on extraction wound healing

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THE EFFECTS OF 17-∝-ETHYL-19-NORTESTOSTERONE ON EXTRACTION WOUND HEALING

BY

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THE EFFECTS OF 17-α-ETHYL-19-NORTESTOSTERONE

ON EXTRACTION WOUND HEALING

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Head of the Major Department

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CHAPTER I

INTRODUCTION

The multitudinous chemical reactions which comprise the living organism are in a state of dynamic equilibrium. The classic experiments of Schoenheimer, Rittenberg, and others, using isotope tracer techniques have furnished ample proof of this. Organs, tissues and their chemical components are continually being broken down and resynthesized. This dynamic state goes on in an organism regardless of disease, fracture or any illness as long as life lasts.

The concept of the "metabolic pool" is well accepted today in the biological vocabulary. There is general agreement that the mixture of fatty acids, amino acids, carbohydrates, vitamins and minerals which exist in living organisms can be called upon to supply material for anabolism. Also, this "pool" of chemicals can be supplied in turn by exogenous food metabolities and by the degradative components of tissue catabolism. According to Arnstein and Neuberger (1) "the continuous flow of metabolic fragments into the organs and tissue from the pool, and degradative components of organs and tissues going to the pool, are, with few exceptions, handled on the basis of first in, first out or last in, last out."

Nitrogen balance is one aspect of the metabolic equilibrium that is of special interest in this study. Nitrogen balance may be defined as the quantitative difference between nitrogen intake and nitrogen output. It is expressed as grams of nitrogen per day. Intake is the nitrogen of the food and output includes excretion of urine, feces,

sweat, as well as loss of hair and skin. A positive nitrogen balance exists when intake of nitrogen exceeds output. This occurs during the growth of the young, or whenever new tissue is being synthesized. Negative nitrogen balance exists when the output exceeds intake. This may occur during fevers, infections and post-operative recovery. Since most of the nitrogen of the diet represents protein, and most of the nitrogen excretory products are derived from protein catabolism, it is apparent that the balance between the two will reveal significant features of protein metabolism.

Nitrogen balance is an important factor in major surgery. It is a well known fact that the nutritional status and general physical health of the patient has an effect on wound healing. As early as 1930, evidence was produced to show the diet was of importance in wound healing. Harvey (6) found that an increase in the protein of the diet favorably affected the velocity of fibroblast growth in the healing wound.

It has been shown that the rate of protein catabolism is increased after an operation (11). When the catabolic period is prolonged significant protein deficiencies may occur. This is particularly true when a patients appetite is poor. Even though the patient is able to increase his intake of protein a catabolic condition is still difficult to alleviate. Thus post-operative patients become deficient in nitrogen followed by a marked decrease in wound healing and rapidity of convalescence.

Homburger (7) states, that in his opinion, "the amounts of protein needed to replenish protein stores may be truly tremendous. Thus

to insure optimal protein repletion in the post-operative phase as much as 0.6 Gm. of nitrogen (3.75 Gm. of protein) per Kg. of body weight per day may be necessary. This is of the order of 200 to 250 Gm. of protein per day in patients of average weight." In contrast to the post-operative patient, studies made by many investigators (9) show that nitrogen balance can be maintained in the normal healthy individual on a consumption of less than 50 Gm. of protein per day.

Best and Taylor (2) indicate that from 300 to 400 Gm. of body protein may be destroyed daily in febrile patients with very severe infection. They also noted that it was impossible to maintain the patient in nitrogen equilibrium by giving liberal allowances of protein which, under ordinary circumstances, would be considered quite adequate for energy purposes. Thus a febrile patient upon a diet which has been considered adequate in the past is actually in a half-starved state, and is, consequently, forced to consume his own tissue.

The importance of adequate protein supply has been demonstrated by Kobak, et al. (10). They reported that "in the presence of serum protein depletion and marked weight loss, the wounds [of the abdominal wall] invariably exhibited a characteristic, although moderate, delay in the early phases of fibroplasia. Wounds of rats maintained on an adequate protein diet usually heal cleanly by primary intention, whereas those of rats feed a low protein ration tend to heal with more wound infections and excess wound secretions." Cuthbertson (3) found that after fracture of a femur, by open operation and without splinting, that rats show a marked disturbance of general metabolism with an increase in catabolism and of protein in particular.

From the preceding material it can be seen that a substance which reverses negative nitrogen balance, and thereby promotes protein tissue synthesis, is of inestimable value in post-operative therapy. Drugs of this type are called "anabolic agents", as they enhance tissue anabolism.

The androgens are protein anabolic agents. In addition they produce certain characteristic masculinizing effects. For example, testosterone is capable of producing a positive nitrogen balance. In recent years a great deal of work has been done to create steroid anabolic agents that possess a low androgenic activity. One of this type of drug is Norethandrolone (Nilevar).[1] Chemically Norethandrolone is 17-alpha-ethyl-17-hydroxy-19-nor-4-androsten-3-one or 17-alpha-ethyl-19-nortestosterone. Norethandrolone is capable of promoting anabolism to a high degree while maintaining a low androgenic activity. Compared with testosterone propionate, Norethandrolone has approximately only one-sixteenth of the androgenic activity, yet is equally potent as an anabolic agent (12).

The benefits of Norethandrolone in counteracting negative nitrogen balance has been demonstrated (11). For example, one such study concerned patients who had undergone major pelvic surgery (11). Those patients injected with Norethandrolone, showed a marked positive nitrogen balance, compared with the consistently negative nitrogen balance among controls given injections of saline.

In another investigation patients with major extremity fractures

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[1] G. D. Searle and Company.

were treated with the anabolic agent (11). In one patient the negative nitrogen balance was not only reversed but positive nitrogen balance increased to 2.3 Gm. per day. In the other case positive nitrogen balance was increased from 3.5 Gm. to 6.4 Gm. per day.

The rapidity of extraction wound healing varies with the type of extraction. However, it also varies with the patient. There is little doubt that the overall nutritional status of the patient has an effect on the healing rate of this type of wound. Protein is essential for the laying down of the ground substance, and connective tissue in general. Since protein anabolic agents are known to affect the rate of protein synthesis, this study was initiated in an effort to determine the effect of a drug of this type on extraction wound healing.

CHAPTER II

STAGES OF THE HEALING PROCESS

Tissue repair may be said to take place in three stages which are more or less intermingled; namely, granulation, organization, and cicatrization. First, repair is evidenced by proliferation of the fibroblasts and capillary endothelium (angioblasts). The formation of new capillaries occurs almost as soon as does proliferation of fibroblasts. The mass of fibroblasts and new capillaries is called granulation tissue, because, when seen in an evacuated abscess cavity, the surface is roughened by the projection of soft red granules which contain the fibroblasts and new capillaries.

Organization differs from simple granulation. The fibroblasts and capillaries replace the exudate and destroyed tissues. In the case of fibrinous exudates, the line of growing fibroblasts and capillaries may follow the meshes of the fibrin net. Also, during the stage of organization, the formation of the collagenous fibrils of the connective tissue becomes noticeable.

When the defect is filled or the exudate replaced by granulation tissue the capillaries decrease in size and for the most part undergo gradual atrophy until they disappear. Simultaneously, the connective tissue shows shrinkage and condensation of the nuclei. The cytoplasm becomes more fibrillar, until the mass takes on the character of mature connective tissue. This transformation is cicatrization.

CHAPTER III

EXTRACTION WOUND HEALING

The healing process following molar extraction in normal male rats has been studied histologically by R. F. Huebsch, et al. (8). Seven hours after extraction they found the socket filled with a blood coagulum. There was an uneven distribution of blood cells in the coagulum. Also, a heavy accumulation of leukocytes and erythrocytes was noted.

Fourteen hours following extraction the fibrin network was the most conspicuous feature in the clot.

Twenty-three hours after the extraction the organization of the clot had begun. This was indicated by the ingrowth of a large number of capillaries in the region of the periodontal membrane.

Three days postoperatively the ingrowth of fibrocytes into the coagulum occurred with bundles of elongated fibrocytes infiltrating the coagulum and forming a young, unorientated connective tissue.

The first indication of bone formation in the socket itself was seen on the fifth day after the extraction. The former periodontal membrane had almost disappeared with the space previously occupied by it being filled with connective tissue of the same appearance as that seen in the rest of the socket.

After ten days of healing, the epithelium had proliferated across the connective tissue and closed the wound. Immature, coarse, fibrillar bone was observed growing into the socket from the side walls of the alveolus. The investigators found that, at this stage, the connective tissue exhibited a more mature structure and a definite orientation of

the cells.

Thirteen days after the extraction the socket was almost filled with new bone, the epithelium resembling that of the surrounding mucous membrane. Twenty-five days after the extraction the entire socket was filled with young bone with the epithelium consisting of a fully differentiated, stratified, keratinized squamous epithelium.

The presence of retained bone and root fragments has been reported to have a definite effect on the healing of extraction wounds. Glickman (5) found that the presence of retained fragments actually delayed healing. Smith (13) studied the role of epithelium in the healing of experimental extraction wounds. The epithelium was found to be the primary agent involved in the elimination of retained bone and root fragments. It was suggested that there is a relationship between retained root and bone fragments, inflammatory exudates and an epithelial response.

Therefore, it would appear that a study of experimental extraction wound healing may be influenced by the completeness of the extraction. The effects of chemotherapeutic agents on extraction wound healing may be altered by the presence of root and bone fragments. Thus it is essential that complete extractions be used for comparison if possible.

CHAPTER IV

EXPERIMENTAL PROCEDURE

Outline of Experiment

A total of twenty-five female, Sprague-Dawley rats ranging in weight from 195-260 gms. were chosen for this study. The maxillary molar of the rat was considered suitable for our purposes, since it is morphologically similar to the human molar.

A period of two weeks was allowed before extraction in order that the animals would become accustomed to their environment and diet. At the end of this time the animals were separated randomly into those to be used for control and those to be treated with the drug. One control and one treated rat were paired to a cage. The animals were maintained on a stock diet. Water was given ad libitum.

Starting one day prior to extraction and continuing daily for a total of 14 days the treated rats were given 1.0 mgm. of 17-alpha-ethyl-19-nortestosterone in 0.04 ml. of a solution of 10% v/v benzyl alcohol in sesame oil.[2] The drug was injected intramuscularly in alternate hind legs. At the same time 0.04 ml. of a solution of 10% v/v benzyl alcohol in sesame oil was injected intramuscularly in alternate hind legs of the control animals daily for a total of 14 days.

On the second day of the drug treatments, the animals were anesthetized with sodium pentobarbital 35 mg./kg. by intraperitoneal

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[2]Supplied through the courtesy of G. D. Searle and Co., Chicago, Illinois.

injection. Then, bilateral extractions of the maxillary first molars were performed.

The animals were sacrificed according to postoperative intervals of from one to twenty-eight days (Table I). At autopsy the heads were removed and the maxilla cut sagittally in the midline. The left and right sides of each maxilla were separated, decalcified and embedded in paraffin. Specimens were cut buccolingually in serial sections and stained with hematoxylin-eosin for histological comparison.

TABLE I. ARRANGEMENT OF EXPERIMENTAL GROUPS

Animal Number	Extent of Extraction	Treated or Control	Postoperative Period in Days
		Group I	
1L-M-1	Incomplete	Treated	1
1R-M-1	Complete	Treated	1
2L-C-1	Complete	Control	1
2R-C-1	Incomplete	Control	1
3L-M-3	Complete	Treated	3
3R-M-3	Partially	Treated	3
4L-C-3	Complete	Control	3
4R-C-3	Complete	Control	3
5L-M-7	Incomplete	Treated	7
5R-M-7	Complete	Treated	7
6L-C-7	Undetermined	Control	7
6R-C-7	Undetermined	Control	7
7L-M-14	Complete	Treated	14
7R-M-14	Complete	Treated	14
8L-C-14	Incomplete	Control	14
8R-C-14	Complete	Control	14
		Group II	
9L-M-14	Incomplete	Treated	14
9R-M-14	Complete	Treated	14
10L-M-14	Incomplete	Treated	14
10R-M-14	Complete	Treated	14
11L-C-14	Incomplete	Control	14
11R-C-14	Incomplete	Control	14
12L-C-14	Incomplete	Control	14
12R-C-14	Incomplete	Control	14
13L-M-28	Complete	Treated	28
13R-M-28	Complete	Treated	28
14L-M-28	Complete	Treated	28
14R-M-28	Complete	Treated	28
16L-C-28	Incomplete	Control	28
16R-C-28	Incomplete	Control	28
17L-C-28	Complete	Control	28
17R-C-28	Incomplete	Control	28
21L-M-42	Complete	Treated	42
21R-M-42	Complete	Treated	42
22L-M-42	Complete	Treated	42
22R-M-42	Complete	Treated	42
23L-M-42	Complete	Treated	42

TABLE I. Continued

Animal Number	Extent of Extraction	Treated or Control	Postoperative Period in Days
23R-N-42	Complete	Treated	42
24L-C-42	Incomplete	Control	42
24R-C-42	Incomplete	Control	42
25L-C-42	Complete	Control	42
25R-C-42	Complete	Control	42
	Group III		
15L-N-28	Incomplete	Treated	28
15R-N-28	Incomplete	Treated	28
18L-C-28	Incomplete	Control	28
18R-C-28	Complete	Control	28
19L-C-28	Incomplete	Control	28
19R-C-28	Complete	Control	28
20L-N-28	Complete	Treated	28
20R-N-28	Complete	Treated	28

Experimental Animals

The Sprague-Dawley strain of rat has been recognized by many investigators as a suitable animal for experimentation. It is a white rat and is noted for its docility. The animals were divided into three groups. The animals in each group were of the same age and approximately of equal weight. Sex differences, were controlled by the choice of only female animals for this study. The animals were maintained on a Purina Dog Chow diet for the duration of the experiment.

This diet contains: meat and bone meal, dried milk albumin, wheat germ meal, ground oat groats, ground yellow corn, ground wheat, soybean oil meal, cereal food crumbs, animal fat (preserved with butylated hydroxyanisole), vitamin B_{12} and antibiotic feed supplement, artificial

coloring, riboflavin supplement, brewers dried yeast, vitamin A feeding oil, D-activated plant sterol, thiamin, niacin, 5% iodized salt, 0.02% manganese sulfate.

An analysis of this diet shows: crude protein, not less than 24.0%; crude fat, not less than 7.0%; crude fiber, not more than 4.0%; N.F.E., not less than 44.0%; ash, not more than 10.0%; moisture, not more than 12.0%. Water was given ad libitum. All animals ate well following extraction and, except for a preliminary period of no gain (and in some, weight loss) all animals showed weight gain. Since the drug is an anabolic agent, the treated animals showed greater weight gain than the controls, Table II.

TABLE II. AVERAGE WEIGHT GAINS OF EXPERIMENTAL ANIMALS DURING NORETHANDROLONE ADMINISTRATION

Number of Days of Drug Administration	Total Number of Animals	Average Weight Change (Gms.)	
		Treated	Control
1	2	0	15
3	2	0	- 8
7	2	21	6
14	19	39.6	11.4

Wounds prepared by the extraction of rat molars appeared to be suitable for this study. Rat molars are morphologically similar to the human molar. Moreover, in contrast to human teeth, the rat molar is available in large numbers and precisely controlled conditions.

It should be stressed, however, that the small size is a disadvantage during the extraction and histologic examination of the wound. The four slender roots and the dense periodontal membrane, as well as the size, make complete extractions of the rat molar difficult.

Extraction Procedure

Positioning of Animals

After the animals had become anesthetized, they were secured to a board in a supine position. By this means the upper jaw lay flat and the maxillary molars were exposed. A larger operatory field was obtained by gently drawing back the lower jaw and tongue.

Anesthesia

In the dose employed, sodium pentobarbital does not act as a general anesthetic to the degree that there is a complete lack of voluntary leg and tongue movement as well as some swallowing. It was decided, however, that supplemental use of ether would create an error in the results. Ether is noted for its irritability to the mucosa and the degree of irritability could not be accurately controlled from one animal to another.

Following the intraperitoneal injection of sodium pentobarbital (35 mgm./kg.) hypnosis was obtained in about 3 minutes. About 15 minutes was allowed as a time interval before extractions were performed. At no time did the animals inflict injury to themselves upon recovery from the anesthetic. Throughout the duration of the experiment, the only wound each animal received was that of the extractions.

Illumination of the Operatory Field

The small size of the rat mouth makes illumination of the operative field without interference to the operator difficult. It was found that this could be satisfactorily accomplished by the use of a focused two-cell flashlight fastened by an adjustable clamp to a ring stand. By proper positioning, a beam of bright light could be directed over the shoulder of the operator and into the open mouth of the rat.

Surgical Procedure

Bilateral extraction of the maxillary first molars was performed on all animals. The extractions were accomplished by loosening the mucoperiosteum both on the buccal and palatial sides of the tooth with modified scalers. The tooth was then luxated by means of scalers' modified so that the tip was semipointed. This instrument was used as an elevator. The tooth was removed by means of mosquito forceps. The forceps was used only for the removal of the loose tooth. Any attempt to remove a partially loosened tooth with the forceps resulted in the crown being broken off with the roots remaining.

No attempt was made to debride the wound of broken bone and tooth fragments.

Although complete extractions were attempted, it should be noted that the four slender roots and the dense periodontal membrane, as well as the size, make complete extraction of the rat molar difficult.

Bleeding was controlled by the use of cotton swabs and in no case was excessive.

Recovery and Survival

Recovery of the animals from the anesthetic and the operation was uneventful. Recovery was slow. However, upon regaining complete consciousness the animals resumed eating and drinking habits. All animals appeared in good health for the duration of the experiment except one animal that apparently died from respiratory failure due to the anesthetic.

Administration of the Drug

The control animals were given daily injections of a 10% v/v solution of benzyl alcohol in sesame oil in 0.04 ml. quantities. Injections were intramuscular and were made in the hind leg. Alternate legs were used each day.

The control solution was prepared by dissolving 10% by volume of benzyl alcohol in sesame oil, U.S.P. The clear solution was placed in a clean, dry glass ampul and sterilized by dry heat for four hours at a temperature of not less than 140 degrees Centigrade. A multiple dose rubber closure, previously sterilized in boiling water for fifteen minutes and dried, was inserted into the ampul immediately after the oven temperature had dropped to 80 degrees Centigrade.

The treated animals were given daily injections of 17-alpha-ethyl-19-nortestosterone in a solution of 10% benzyl alcohol v/v in sesame oil U.S.P. The daily dose of the drug was 1 mgm./0.04 ml. given intramuscularly into the muscle of alternate hind legs. The drug was supplied in sterile 1 ml. ampuls and upon opening, a multiple dose sterilized rubber closure was inserted to maintain sterility.

Needles and syringes used for the injections were all sterilized

by dry heat for two hours at a temperature of 140 degrees Centigrade.

Sacrifice

The animals were sacrificed at the end of the experimental period listed in Table I. This was accomplished by administration of an overdose of sodium pentobarbital intraperitoneally. In order to obtain a nearly bloodless specimen, blood was removed by cardiac puncture before cardiac arrest occurred.

The maxillary portion of the head was removed after death by making incisions from the mouth through each cheek and then cutting laterally through the skull in the region of the ears. After photographs of the wound area had been taken, an incision was made sagittally in the midline of the palate. The right and left portions of the maxilla were dissected from the rest of the tissue.

Histological Technique

Fixation

Immediately after sacrifice the maxillary tissue was trimmed into pieces no more than 5 mm. in thickness to permit rapid and complete penetration of the various mediums encountered throughout the histological process. The tissue was washed in saline solution to remove foreign materials. Care was exercised not to damage the wound area. A solution of glacial acetic acid, 2 ml.; commercial formalin, 10 ml.; and 70 percent alcohol, 90 ml., was employed as the fixative reagent. Each piece of tissue was placed in a 50 ml. cork stoppered Erlenmeyer flask containing the fixative reagent for a period of 24 hours. All corks were covered

with aluminum foil to prevent contamination. Similar flasks were employed throughout the process leading to embedding of the tissue.

Decalcification

Since the wound area contains considerable amounts of bone, decalcification was necessary before proceeding with the histological process. This was accomplished by means of a solution containing 70 percent alcohol, 97.5 parts; and concentrated nitric acid, 2.5 parts. In some instances the solution had to be changed frequently until the tissue was decalcified. Each day, in order to determine the extent of decalcification, a small amount of the tissue which was not in the wound area was sliced with a razor blade. From four to seven days was required for complete decalcification.

Following decalcification each tissue was mordanted for two hours in a freshly prepared two percent aqueous solution of potassium alum before being washed in distilled water. The washing process continued for 20-24 hours.

Dehydration

The purpose of dehydration is to remove the water which the tissue contains. This must be done with a medium which will replace the water and which will occupy the space left by it. The medium must be miscible with the clearing agent, and not undo the work of the fixing agent, as for example softening the tissue. Since absolute alcohol fulfills the preceding requirements it was employed in the following manner.

The tissue was passed through a graded series of alcohols of

increasing concentration, beginning with 50 percent and going through 70, 80, 95 percent, finally to absolute alcohol. The tissue remained in the 50 and 70 percent solutions for 30 or 45 minutes. It was left in the 95 percent solution and in the absolute alcohol for one hour. The tissue was preserved indefinitely in the 80 percent alcohol until it was convenient to proceed with the remainder of the process. Eighty percent alcohol is the only stopping point in this process until the tissue is embedded in a paraffin block.

Clearing

By this process the alcohol of dehydration is displaced by a medium which is miscible with alcohol as well as with paraffin. We encountered a great deal of difficulty in this process using xylol as the clearing agent. Although xylol fulfills the requirements of a clearing agent it causes the tissue to become hard and brittle thus impeding sectioning. Often the results were damaged beyond use. To correct this undesirable situation dioxane was substituted for xylol with satisfactory results.

From absolute alcohol the tissue was transferred to a mixture of equal parts dioxane and absolute alcohol and remained for two hours. Then the tissue was placed in dioxane for two hours.

Infiltrating

The purpose of this process is to permit the embedding medium to infiltrate into all parts of the tissue.

The tissue was transferred to a mixture of equal amounts of dioxane and paraffin (melting point 53-55°C) for one hour. Then it was

placed in a primary melted paraffin bath for two hours. Following this it was transferred to a second melted paraffin bath for two hours. While in the first paraffin bath the tissue was moved frequently. The dioxane, being replaced within the tissue by the paraffin, forms a small layer around the lower area of the tissue. Thus, it inhibits, to a degree, complete infiltration of the paraffin if this agitation of the tissue is not done.

Embedding

To embed the tissue in a paraffin block a small cup, approximately one cubic centimeter, was formed using aluminum foil. The cup was set on a level place near the melted paraffin bath. Then paraffin, melted with the aid of a water bath, was poured into the cup until it was level full. The tissue was transferred, wound area down, with the aid of slightly heated forceps, to the cup and quickly orientated into position. The cup was held in cold water until the paraffin hardened, then placed in a refrigerator for storage.

At all stages it is important that the paraffin be kept at the lowest temperature possible, since high temperatures cause a hardening of the tissues.

Sectioning

The layer of aluminum foil was removed and paraffin block trimmed to a neat rectangular form. The block was secured on the object disc, which had a thin layer of paraffin, by heating the disc slightly and pressing the block and disc together. Before sectioning was attempted, the disc was allowed to cool.

Sectioning of each block was accomplished with a rotary microtome. The tissue block was cut into 10 micron strips so that the wound area was cut buccolingually. On cutting, the paraffin blocks yielded ribbons of sections which were easily stored in flat paper boxes.

Mounting

Water at a temperature below the melting point of paraffin was added to a flat, clean dish. A section of tissue from the ribbon was transferred, dull surface up, to the water. A small drop of Mayer's albumen was placed on a clean slide and spread evenly over the area of the slide to be occupied by the section. Then the slide was lowered into the water containing the tissue slice and the tissue brought up over the albumenized area. A needle was used to hold the tissue while the excess water was drained from the slide. The slides were then placed on a constant temperature drying tray until dried sufficiently for the staining process.

Staining

The tissue was stained with hematoxylin-eosin. As Harris hematoxylin solution is aqueous, it was necessary to again hydrate the sections. Before this could be accomplished the paraffin was dissolved using a solution of xylol. From xylol the sections were gradually diluted down to water utilizing various graded solutions of alcohol.

Coplin jars were filled with solutions in the following order: xylol, xylol-alcohol 50:50, absolute alcohol, 95 percent alcohol, 80 percent alcohol, 70 percent alcohol, 50 percent alcohol, distilled water. The slides were transferred, one at a time in definite order,

successively through the series to water. Then they were placed in the Hematoxylin solution for three to four minutes depending upon the extent of drying. Hematoxylin functions as a stain for the nuclear structures within the tissue. The slides were then transferred to slightly alkaline tap water which differentiated or "blued" the stain in the section.

An alcoholic solution of Eosin Y was used as a counterstain for the sections. Cytoplasm and connective tissue fibers stain red with eosin. As the eosin dye is a solution in 90 percent alcohol, the slides were transferred to a 70 percent alcohol solution previous to counterstaining. After less than one minute in the counterstain the slides were transferred successively to 70 percent alcohol, 80 percent alcohol, 95 percent alcohol, absolute alcohol, xylol-absolute alcohol 50:50, and xylol. This was necessary in order to "clear" the slides previous to permanent mounting.

Permanent mounts were then prepared by placing a solution of one part xylol to two parts of Canada balsam on the slide and afixing a cover slip.

The slides were examined for histological comparison of cellular growth.

CHAPTER V

EXPERIMENTAL RESULTS

A description of the histological characteristics of representative extraction wounds follows. The results are grouped according to each post-operative time period.

Twenty-four Hour Samples

The wound areas in all samples, in both treated and control animals, show the alveolus filled with debris. The debris is composed mainly of a fibrin network. Enmeshed in the fibrin are both erythrocytes and leucocytes. There was no evidence of granulation or organization in any of these samples. No distinction between the wound areas of treated and control animals was evident. (Figures 1 and 2)

Three Day Samples

Treated Animals

The alveolus contains numerous bone chips, although it was thought at the time of operation that extraction was complete. Organization of the wound is almost complete with fibroblasts and macrophages filling all but the marginal portion of the wound area. (Figure 3) A magnification of the wound area shows the infiltration of connective tissue cells into the fibrin. (Figure 4) Outgrowth of osteoblasts resulting in new bone formation can also be seen. The epithelium has started to proliferate across the wound. (Figure 3)

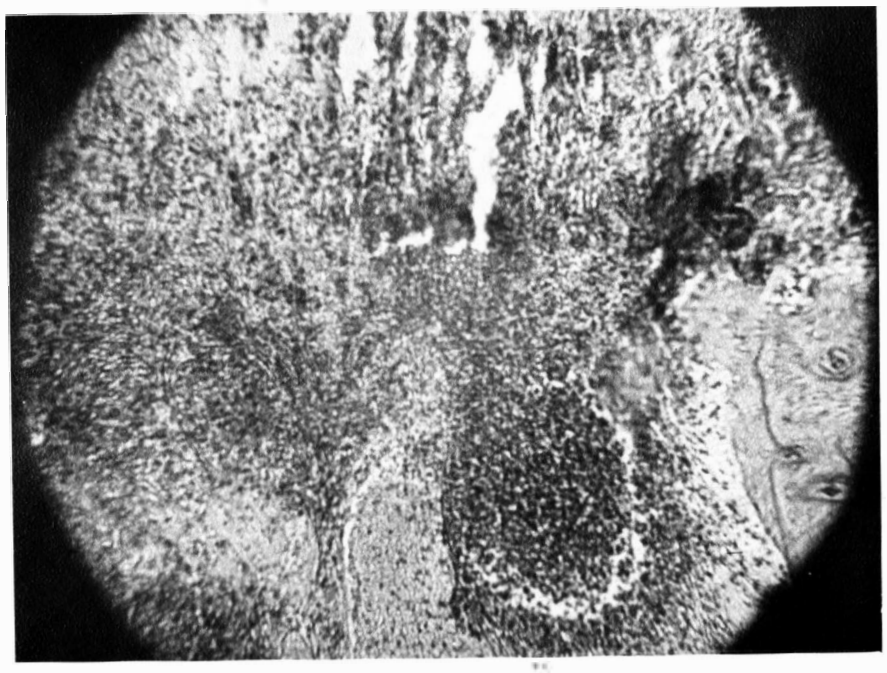

Figure 1. Twenty-four Hours After Extraction (Animal No. 1R-N-1, X125).

Figure 2. Twenty-four Hours After Extraction (Animal No. 2L-C-1, X125).

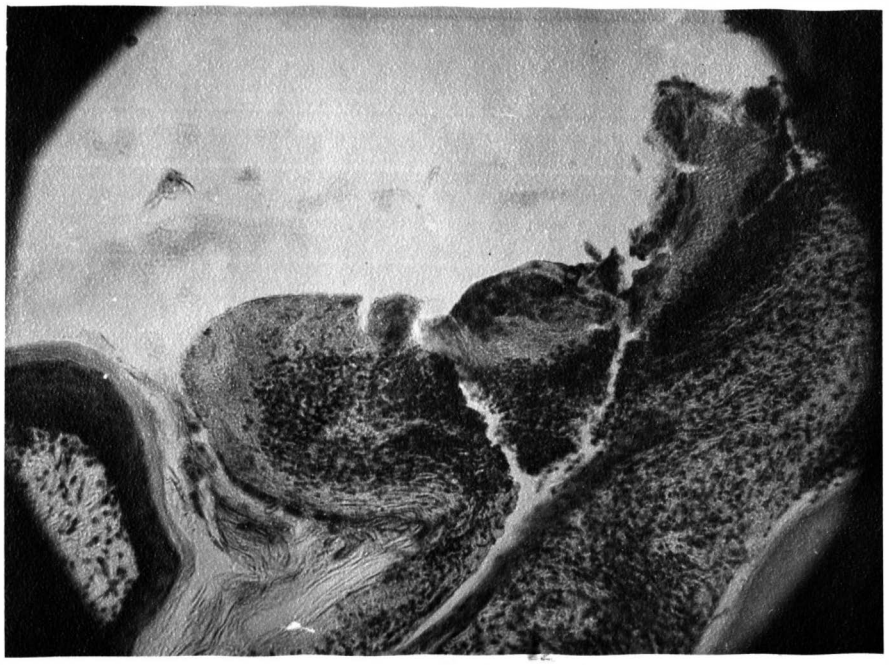

Figure 3. Three Days After Extraction (Animal No. 3L-N-3, X125).

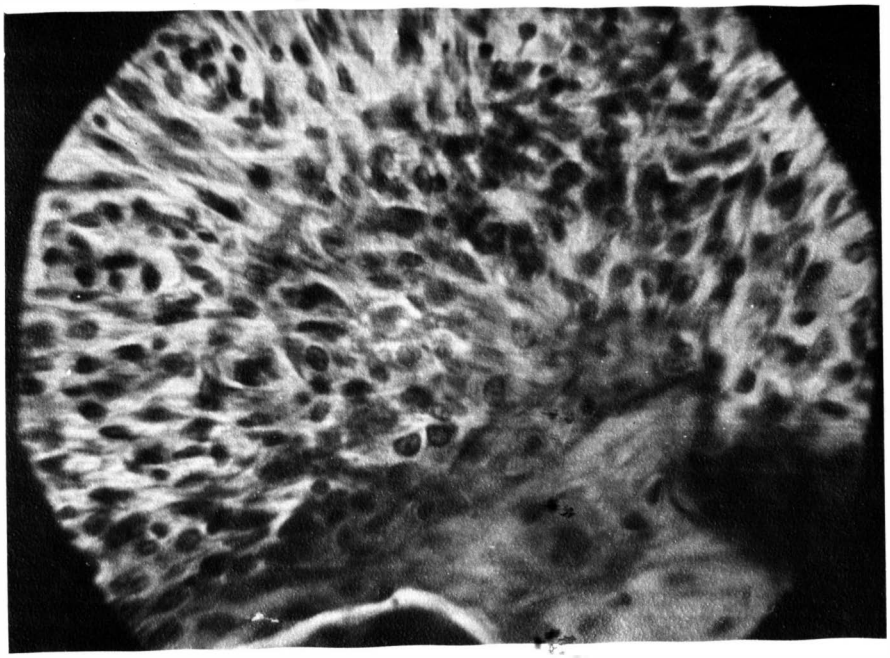

Figure 4. Three Days After Extraction (Animal No. 3L-N-3, X537).

Control Animals

Proliferation of the epithelium has started to seal off the wound. (Figure 5) Extensive fibrin formation and debris with large masses of leucocytes can be noted. (Figure 6) Several fragments of crown and roots are present. Fibroblasts and macrophages are starting ingrowth into the coagulum forming young connective tissue. It appears that new bone formation may be starting at the bottom of the alveolus and along the buccal aspect of the alveolar bone. The periodontal membrane appears to be degenerating. The fibrin area does not show any evidence of organization, since there is no growth of capillaries in this area.

Seven Day Samples

Treated Animals

The epithelium can be seen migrating across the wound area from the buccal aspect of the alveolar bone. (Figure 7) Organization of the tissue is progressing and numerous capillaries filled with red blood cells can be seen. The young connective tissue does not show orientation of the long axis of the cells. New bone formation is taking place in the marrow spaces adjacent to the socket and the bottom of the alveolus.

Control Animals

Figure 8 shows that the majority of the new tissue within the wound area is organized. Fibroblasts and macrophages are present along with a well-developed capillary system. As in the treated animals; the new connective tissue cells are unoriented as far as similarity of the long axis of the cells is concerned. Several tooth chips were noticed and a

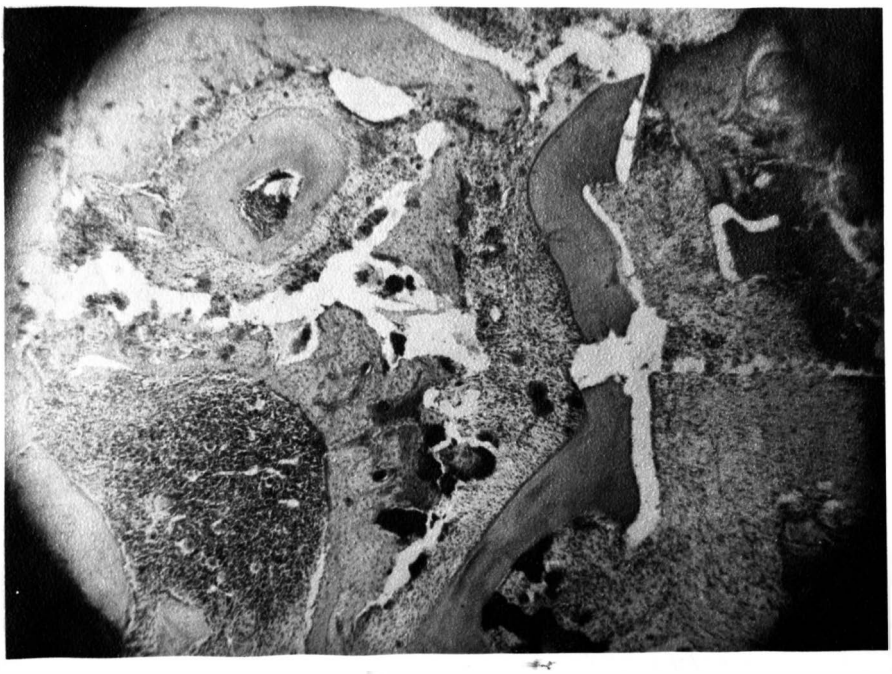

Figure 5. Three Days After Extraction (Animal No. 4L-C-3, X43.7).

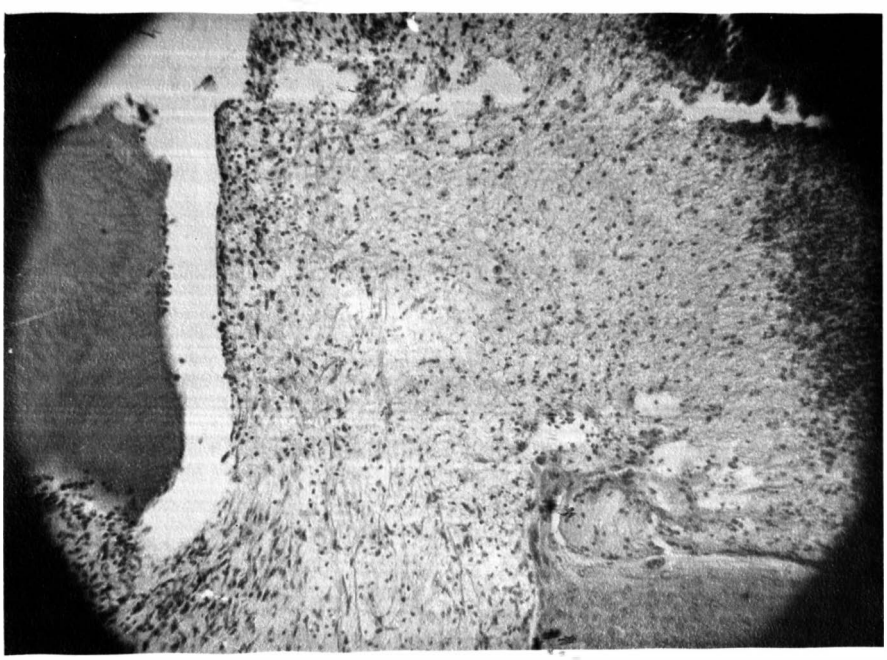

Figure 6. Three Days After Extraction (Animal No. 4L-C-3, X125).

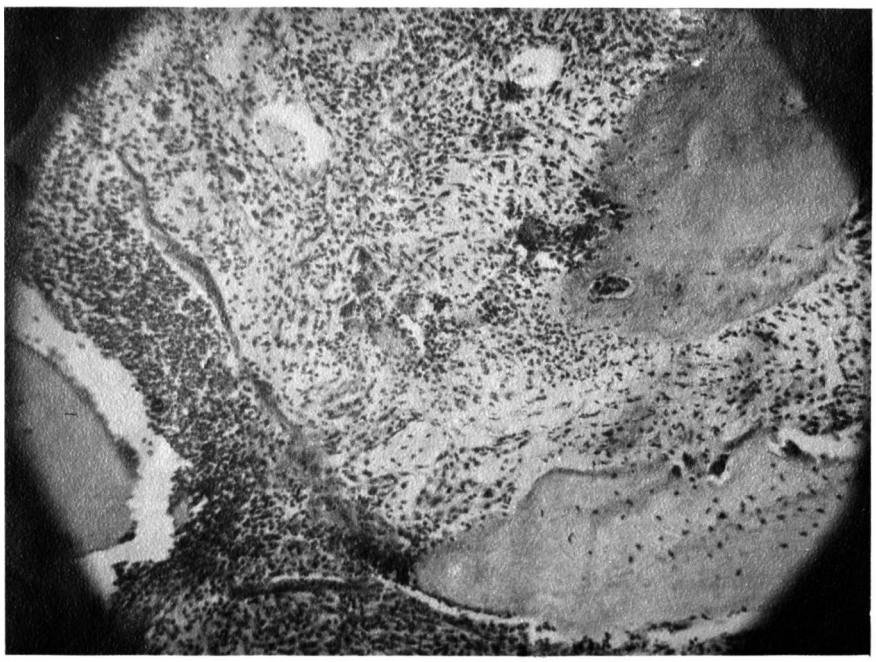

Figure 7. Seven Days After Extraction (Animal No. 5L-N-7, X125).

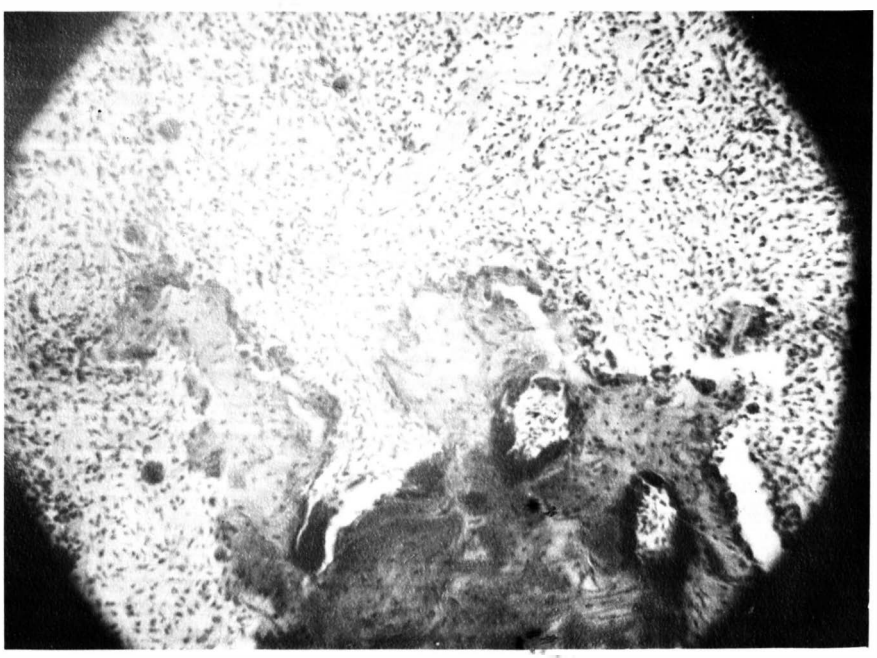

Figure 8. Seven Days After Extraction (Animal No. 6L-C-7, X125).

region of unorganized tissue was present in the marginal area of the wound. The buccal alveolar crest shows signs of resorption. [Howships' lacunae] .

Fourteen Day Samples

Difficulties were encountered with the decalcification process in the preparation of histological sections of these samples. For this reason there were no slides available for the comparison of wound healing at this time-interval following extraction.

Twenty-eight Day Samples

The wound areas in all samples, both treated and control animals, are similar in almost every respect. Young bone has taken the place of the early connective tissues. (Figure 9) The bone forms a straight line buccolingually in the marginal area. The new bone formations are covered with a layer of osteoblasts.

The connective tissue layer is comprised of mature connective tissue cells and a definite orientation of the cells can be noted. The direction of the long axis of the cells is mainly horizontal to the epithelial layer. There are few capillaries present indicating that healing has reached the stage of cicatrization. The epithelium consists of a fully differentiated, stratified, keratinized, squamous epithelium. The polyp-like characteristics of the lamina propria as seen in all late postoperative periods can be noted.

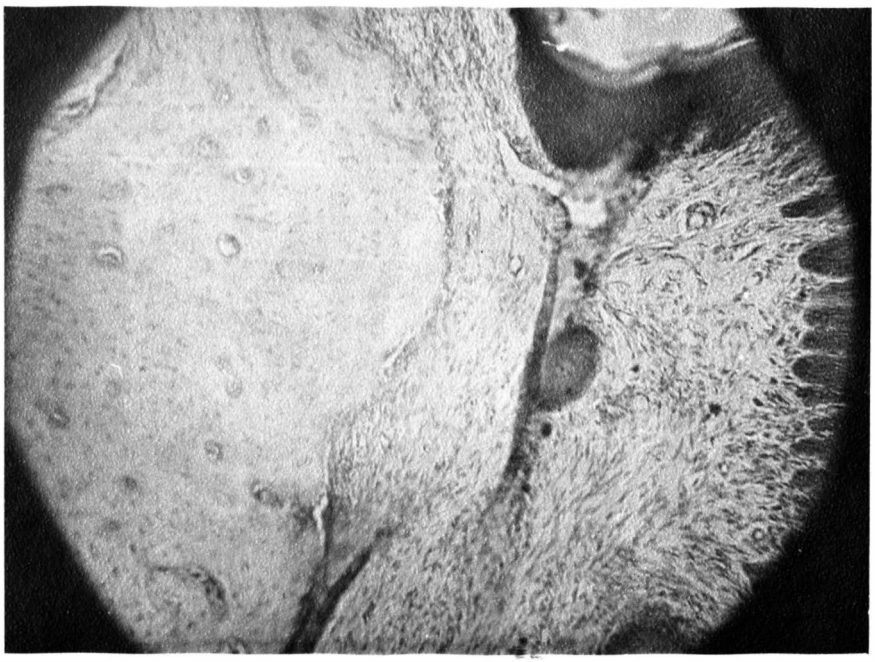

Figure 9. Twenty-eight Days After Extraction (Animal No. 14L-N-28, X125).

Forty-two Day Samples

The majority of these healed extraction wounds could not be distinguished from those of the 28 day period.

CHAPTER VI

DISCUSSION

Norethandrolone, in the dose given, exhibited anabolic effect on rat growth. It can be noted from Table II that the treated animals gained an average of 39.6 Gm. per animal, during the 14 days of drug administration, compared to 11.4 Gm. for the controls. The dose of Norethandrolone (one mgm./rat/day) and the administration of this dose over a 14 day period, where possible, was based on studies by Drill and Saunders (4). Growth is evidence of positive nitrogen balance in the treated animals. It would appear that the dose employed is sufficient to produce growth stimulation of extraction wound tissue if this is an effect of the drug.

In only one case, after histological analysis of the wound areas, can it be said that the treated animals show greater healing per unit of time than the control animals. The three day samples did show a slight, but noticable difference. The sample of the treated animal showed definite organization of the wound area. The fibrocytes filled all but the marginal portion of the wound. In the sample from the control animal, organization was not noted although the fibrocytes had started ingrowth into the coagulum.

Huebsch (8) found that organization begins twenty-three hours after extraction as indicated by capillary ingrowth. He also found that the fibrocytes invade the socket approximately three days after extraction to form the young connective tissue. Based on the extent of fibrocyte invasion, it would appear that our three day sample from the treated

animal was further advanced than that of Huebsch. This is even more notable in view of the presence of bone fragments.

There is no doubt that the extensive bone and tooth fragments left in some of the extraction wounds interferred with the healing process. This has been amply proven by others (5) (13). We have noted that those 42 day post-extraction samples containing bone fragments were not as completely healed as the 28 and 42 day samples which were free of this type of debris.

Based on our observations it would appear that a further study of this type is warranted. These further studies should be concentrated on evaluation of tissue growth at shorter post-operative time periods. It is possible that sacrifices at daily intervals up to two weeks after extraction would constitute valuable samples for study. Also the extreme difficulty that is encountered in the extraction process, in order to obtain complete extractions, may dictate the use of a larger number of experimental animals.

CHAPTER VII

CONCLUSIONS

1. A histological study has been made of extraction wound healing in rats treated with Norethandrolone compared with extraction wound healing in control animals.
2. In the majority of the wounds analysed, there does not appear to be a difference in the rate of healing between treated and control animals.
3. An apparent difference in the rate of healing of one experimental group was noted at the end of a three day post-extraction period. The treated animal appeared to be further advanced in extraction wound healing than the control.
4. It would appear feasible to restudy this experiment using a larger group of experimental animals and making comparisons at shorter time intervals.

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